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# Evidence for Resistance to *Bacillus thuringiensis* (*Bt*) subsp. *kurstaki* HD-1, *Bt* subsp. *aizawai* and Abamectin in Field Populations of *Plutella xylostella* from Malaysia

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**Abstract:** The efficacy of *Bacillus thuringiensis* (*Bt*) subsp. *kurstaki* HD-1 ('Dipel'®; *Btk;* CryIA & CryII) and *Bt.* subsp. *aizawai* ('Florbac'®; *Bta;* CryIA & CryIC) was assessed against larvae from various field populations of *Plutella xylostella* (F2 generation) collected in the Cameron Highlands, Malaysia in April 1994 and a lowland population (SERD 2; F10 generation) collected in December 1993. Evidence of resistance to *Btk* and to a lesser extent *Bta* is reported in these populations ($LC_{50}$ Toxicity Ratios [TR] = 3–14 and 2–8 respectively), most notably in SERD 2. The first recorded evidence of resistance to abamectin (TR = 17–195-fold) in field populations of *P. xylostella* is also reported. In an unselected sub-population of SERD 2, the TR values for *Btk*, *Bta* and abamectin declined 2- to 3-fold ($P < 0{\cdot}01$) over six generations in the laboratory (F10–F16) while in sub-populations of SERD 2 selected with these products (F11–F15) there was a significant ($P < 0{\cdot}01$) increase in the TR (15-, 3- and 2·5-fold respectively) when compared with the F10 generation. This suggests the presence of marked resistance to *Btk* and some resistance to *Bta* and abamectin. There is also evidence of slight cross-resistance to *Btk* in the *Bta*-selected sub-population but no evidence for the reverse selection of resistance or for cross-resistance between *Btk* and abamectin. Concurrent selection studies (F11–F15) with another sub-population of SERD 2 demonstrated resistance to the acylurea insect growth regulator, teflubenzuron ('Nomolt'®) (29-fold increase in TR). Based on the selection experiments with SERD 2, estimates of realised heritability ($h^2$) of resistance gave very high values for teflubenzuron and *Btk* (*c.*0·7) and moderate values for abamectin and *Bta* (*c.*0·3). The results are discussed in relation to integrated pest management (IPM) and insecticide resistance management (IRM) strategies for *P. xylostella.*

Key words: *Plutella xylostella*, insecticide resistance, avermectin, acylurea, teflubenzuron

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## 1 INTRODUCTION

The difficulties experienced in controlling the diamondback moth, *Plutella xylostella* L. on cruciferous crops in Southeast Asia and other areas worldwide over the past 30 years have been due largely to the over-use of pesticides. This has led both to the development of resistance to successive groups of compounds and to the destruction of natural enemies.[1–3] Over-use of pesticides has also resulted in increasing concern over pesticide residue levels and this has provided the main impetus for the development of integrated pest management (IPM) systems for *P. xylostella* and other crucifer pests.[1]

Most IPM systems have included the release and/or conservation of parasitoids of *P. xylostella*.[1] Biological control involves an overall reduction in pesticide use and a change wherever possible to the application of more selective insecticides, for example, acylurea insect growth regulators, abamectin (avermectin $B_1$) and *Bacillus thuringiensis* Berliner (*Bt*) products containing crystal (Cry) protein endotoxins.[4]

Products based on *Bt* are widely regarded as being the least harmful to natural enemies of the currently available classes of chemical pesticides.[4] They were used, at relatively low volumes, for more than 20 years before significant levels of resistance were observed in field populations of any pest species.[5] Field resistance to *Bt* still appears to be limited to a few insect crop pests and has only been fully substantiated in *P. xylostella*.[5] However, the widespread and increasing number of field populations of *P. xylostella* exhibiting resistance to *Bt*[5,8] is a serious threat to the use of such products and their replacement by less selective compounds could be highly detrimental to biological control systems. The prospective rapid rise in the use of *Bt* in agriculture through the application of new strains and the expression of Cry genes in transgenic bacteria and crop plants[7] is expected to increase selection pressure for resistance in a variety of insect pests unless appropriate management strategies are adopted.[5,6]

Resistance to *Bt* subsp. *kurstaki* (*Btk;* containing CryIA(a,b,c), CryIIA & CryIIB toxins) and to a lesser extent *Bt* subsp. *aizawai* (*Bta;* containing CryIA(a,b), CryIC & CryID toxins) in field populations of *P. xylostella* has been observed in various countries.[5] In Malaysia, resistance to *Btk* was reported in populations of *P. xylostella* from the Cameron Highlands and the lowlands in 1990.[8] Interviews with pesticide retailers and farmers in the Cameron Highlands in March–April 1994 suggested that many local farmers had complained of poor efficacy with *Bt* products and that only formulations containing both of the above *Bt* subspp. (e.g. 'Turex'®) retained efficacy in all situations (Verkerk, R. H. J., unpublished results). In addition, there were also complaints of reduced efficacy with abamectin, (Verkerk, R. H. J., unpublished results) a product for which there are very few published reports of 'field' resistance in agricultural pests.[9,10]

In the present study, the level of resistance to *Btk*, *Bta* and abamectin was estimated in populations of *P. xylostella* from the three major geographical zones of the Cameron Highlands and in a lowland population (SERD 2) from Serdang, Selangor State, near Kuala Lumpur. Subsequent insecticide selection studies on SERD 2 investigated the potential for resistance and cross-resistance to *Bt* products and abamectin. The pattern of resistance to *Btk* and *Bta* was of particular interest since reports to date suggest that resistance to *Btk* in field populations of *P. xylostella* is associated with loss of binding of specific Cry toxins to midgut receptors.[5] The status of resistance in SERD 2 to the acylurea insect growth regulator teflubenzuron, a product widely used in Malaysia during the 1980s,[8] was also examined.

## 2 EXPERIMENTAL METHODS

### 2.1 Chemicals

*Bt* subsp. *kurstaki* HD-1 ('Dipel'®), 16 000 iu $mg^{-1}$ wettable powder (WP), and *Bt* subsp. *aizawai* ('Florbac'), 8500 iu $mg^{-1}$ suspension concentrate (SC), were stored in the dark at 4°C. Abamectin ('Agrimec'®), 18 g $litre^{-1}$ emulsifiable concentrate (EC) and teflubenzuron ('Nomolt'®), 50 g $litre^{-1}$ suspension concentrate (SC) were stored in the dark at room temperature. Test dilutions of formulated pesticides were freshly prepared in distilled water with 'Triton' X-100 (50 $\mu g\ ml^{-1}$) as an additional wetting agent.

### 2.2 Insects

Six field populations of *P. xylostella* from Malaysia were examined: five Cameron Highland populations collected in April 1994 from Habu [Kebun Ramasamy] (southern zone), Tanah Rata [MARDI farm] and Mensum Valley [Kebun Chai] (central zone) and Kuala Terla [Kebun Rama] and Kampung Raja [Kebun Chow] (northern zone); and a lowland population (SERD 2), collected originally from the Serdang Campus, Universiti Pertanian Malaysia, Selangor State in December 1993 and cultured in the UK from F7. Two unselected laboratory populations of *P. xylostella*, 'SS' (originally from Asian Vegetable Research & Development Centre, Taiwan, maintained at MARDI, Tanah Rata for *c.*30 generations) and Rothamsted ('ROTH', maintained at Rothamsted, UK for >60 generations), with no apparent resistance to insecticides were used for comparative purposes in Malaysia and UK respectively.

In Malaysia, all insect populations were cultured and tested at ambient temperature (*c.*18–23°C) on foliage from outdoor, organically grown common cabbage (*Brassica oleracea capitata* L., cv. KY-Cross). In the

UK, all insect populations (in each case from an initial population of 150–200 Insects) were cultured and tested at 20(±2)°C and 65(±3)% RH under a 16 : 8 h light : dark cycle on foliage from greenhouse, organically grown four- to six-week-old Chinese cabbage (*Brassica chinensis* Juslen cv. TipTop). In both cases, adult moths were fed on a 50% (w/w) honey solution and larval instars of *P. xylostella* were identified by the width of the head capsule.[11]

### 2.3 Leaf-dip larval bioassays

Insects were assayed on leaf discs (4·8 cm dia.) cut from the centre of the middle leaves of four- to six-week-old cabbage plants using a metal hole punch. Each leaf disc was immersed in the test dilution (Section 2.1) for 10 s, drained on filter paper for 10 s and then allowed to dry on a corrugated sheet of aluminium foil, with the underside of the leaf uppermost, for 1–2 h at room temperature. Control leaf discs were immersed in distilled water containing 'Triton' X-100 (50 $\mu g\ ml^{-1}$). The leaf discs were then placed in individual plastic Petri dishes (5 cm dia.) containing a single, moistened, Whatman No. 1 filter paper (4·5 cm dia.). Five 24 to 48-h-old second-instar (with abamectin and teflubenzuron) or third-instar larvae (with *Bt* products; to comply with standard practice[3]) of *P. xylostella* were placed on each leaf disc ($n = 30$ or 50 per treatment except in discriminating dose tests, Section 2.4). After five days, the remains of the leaf discs were removed and replaced by fresh, untreated cabbage leaves which were then changed *ad lib.* Preliminary experiments were conducted to estimate end-point mortality (time at which mortality in treated population stabilised with no corresponding increase in control mortality) and concentration range suitable for each pesticide. End-point mortality was subsequently taken to be day 9 for *Bt* products and abamectin, and day 13 for teflubenzuron.

### 2.4 Discriminating dose (resistance) test

Larvae (L3) from the F2 generation of each Cameron Highlands field population of *P. xylostella* were assayed (Section 2.3; $n = 30$, 60 or 90 per treatment) in Malaysia, together with larvae from the SS laboratory strain, against *Btk* and *Bta* using the estimated $LC_{95}$ dose for each product against the SS strain. (See Table 1).

### 2.5 Initial log dose–mortality studies

Larvae from the F3 generation of the Habu, Tanah Rata and Kuala Terla (Cameron Highland) populations, the F10 generation of the SERD 2 (lowland) population, and the ROTH laboratory population of *P. xylostella* were assayed (Section 2.3) in the UK against *Btk*, *Bta* and abamectin. Toxicity ratios (TR) for each field population were estimated by dividing the $LC_{50}$ values for a particular compound by the comparable $LC_{50}$ value for the ROTH population (SS strain unavailable in UK).

### 2.6 Laboratory selection studies and cross-resistance tests

The SERD 2 population was assayed (F10) in the UK against teflubenzuron (in addition to *Btk*, *Bta* and abamectin; Section 2.5), then divided into five sub-populations, one of which was left unselected and the others selected with the above insecticides for five generations (F11–F15). A total of 300 larvae were selected per insecticide per generation. Initially (F11), a dose approximating to the $LC_{50}$ for each insecticide against SERD 2 (F10) was used to select for resistance. The doses were adjusted where necessary in subsequent generations, depending upon the percentage adult survival obtained in the previous selection. The average survival rates (F11–F15) were 51, 49, 52 and 50% for *Btk*, *Bta*, abamectin and teflubenzuron respectively.

The unselected and selected sub-populations of SERD 2 were subsequently bioassayed at F14 and F16 (and F19 for abamectin), i.e. after three and five selections respectively. Toxicity ratios (TR) for each sub-population were estimated (Section 2.5) in relation to the ROTH population and the equivalent generation of the unselected sub-population of SERD 2.

An estimate of realised 'heritability' ($h^2$), the proportion of phenotypic variance accounted for by additive genetic variation,[12] was calculated according to the procedure described by Tabashnik[13] in order to quantify, and thus compare, the rates of selection of insecticide resistance in different sub-populations of *P. xylostella*.

In cross-resistance studies (at F16), the *Btk*-selected sub-population was assayed against *Bta* and abamectin, the *Bta*-selected sub-population was assayed against *Btk*, and the abamectin-selected sub-population was assayed against *Btk*.

### 2.7 Statistical analysis

Where necessary, bioassay data were corrected for control mortality.[14] Control mortality did not exceed 10% and was usually 0%. Estimates of $LC_{50}$ values and their 95% fiducial limits (FL) were obtained by maximum likelihood logit regression analysis in GLIM (GLIM 3.77—Numerical Algorithms Group, Oxford, 1985) using generalised linear modelling techniques[15,16] from which differences between data sets were extracted by analysis of deviance. Where the chi-square value indicated heterogeneity, the data were discarded. The $LC_{50}$ values for different treatments were compared at the 1% significance level using individual 95% FL for two parameters.

TABLE 1
Discriminating Dose[a] Leaf Disc Bioassay[b] for *Bt* subsp. *kurstaki* HD-1 and *Bt* subsp. *aizawai* against Third-Instar Larvae of Five Cameron Highland Field Strains of *Plutella xylostella* collected in April 1994 (Assay at F2)[c]

| | *Mortality* (%)[d] | |
|---|---|---|
| *Strain* (*zone*) | Bt *subsp.* kurstaki (*1·6 iu mg*$^{-1}$) | Bt *subsp.* aizawai (*7·03 iu mg*$^{-1}$) |
| Habu [HB] (south) | 42 | 90 |
| Tanah Rata [TR] (central) | 40 | 93 |
| Mensum Valley (central) | 50 | 99 |
| Kuala Terla [KT] (north) | 31 | 93 |
| Kampung Raja (north) | 73 | 96 |
| SS Laboratory strain | 100 | 100 |

[a] Based on *c*.$LC_{95}$ dose for SS strain; $LC_{50}$ values (±95% FL) and slopes (±SE) for *Btk* and *Bta* = 0·09(0·002–0·18) and 2·78(±0·90), and 0·50(0·37–0·64) and 2·77(±0·33) respectively. (Ong, P.C; unpublished results).
[b] Host plant leaf discs: *Brassica oleracea capitata.*
[c] Assay carried out in MARDI Tanah Rata Field Station Entomology Laboratory, Cameron Highlands, Malaysia.
[d] Mortality assessed at day 9; control mortality = 0% ($n$ = 30–90).

## 3 RESULTS

### 3.1 Discriminating dose (resistance) bioassays for *Bt* subspp. *kurstaki* and *aizawai* against field populations of *Plutella xylostella*

In all five of the Cameron Highlands field populations of *P. xylostella* tested, percentage larval mortality was markedly less following treatment with *Btk* than with *Bta* (Table 1). In contrast to the field populations, the SS strain recorded 100% mortality.

### 3.2 Dose responses of field populations of *Plutella xylostella* to *Bt* subspp. *kurstaki* and *aizawai* and abamectin

For *Btk*, the $LC_{50}$ values were significantly ($P < 0·01$) greater in the Tanah Rata, Habu and SERD 2 populations (TR = 7- to 17-fold) than in the ROTH population (Table 2), while for *Bta*, all of the field populations were significantly ($P < 0·01$) different from the ROTH population (TR = 2–8). The $LC_{50}$ values obtained for the ROTH laboratory population (Table 2) were two-fold less with *Btk* ($P > 0·01$) and five-fold less with *Bta* ($P < 0·01$) than the reported values for the SS laboratory population (Table 1). The $LC_{50}$ values for abamectin were significantly ($P < 0·01$) greater in each of the field populations than in the laboratory (ROTH) population (TR = 17 and 195 respectively), this being most marked in the Kuala Terla population from the Cameron Highlands (Table 2).

### 3.3 Stability and selection of insecticide resistance in the SERD 2 population of *Plutella xylostella*

In laboratory culture, the susceptibility of the unselected sub-population of SERD 2 increased ($P < 0·01$) 2- to 3-fold (with a corresponding decline in $LC_{50}$ value) to *Btk*, *Bta* and abamectin over six generations (F10–F15) (Table 3). Selection of sub-populations of SERD 2 with *Btk*, *Bta* and abamectin over five generations (F11–F15 and tested at F16, significantly ($P < 0·01$) increased the $LC_{50}$ value for each product (43-, 8- and 8-fold respectively) compared with the unselected SERD 2 population at F16 (Table 3).

The *Bta*-selected sub-population of SERD 2 had a significantly ($P < 0·01$) greater $LC_{50}$ value (*c*.4-fold) for *Btk* compared with the equivalent value for *Btk* in the same generation (F16) of the unselected sub-population (Table 3), while the $LC_{50}$ value for *Bta* in the *Btk*-selected sub-population was two-fold lower ($P > 0·01$) than the equivalent $LC_{50}$ value for *Bta* in the unselected sub-population (Table 3). The $LC_{50}$ values for abamectin and *Btk* in the *Btk*- and abamectin-selected sub-populations respectively were almost identical to those of the equivalent (F16) unselected sub-population.

In a concurrent laboratory study, the $LC_{50}$ value for teflubenzuron with the unselected sub-population of SERD 2 decreased *c*.two-fold over six generations (F10–F16), although this change was not significant ($P > 0·01$; Table 4). Selection of a sub-population of SERD 2 with teflubenzuron over five generations (F11–F15), significantly ($P < 0·01$) increased the $LC_{50}$ value for teflubenzuron (48-fold compared with unselected SERD 2 population at F16).

Estimations of realized heritability ($h^2$) of resistance for the five laboratory-selected (F11–F15) sub-populations of SERD 2 gave very high values[13] for teflubenzuron and *Btk*, and lower, almost identical values for *Bta* and abamectin (Table 5). This pattern was mirrored by the values for the response to selection ($R$), in this case a function of $h^2$ and the selection differential ($S$). The selection intensity ($i$) was similar for each population (Table 5).

## 4 DISCUSSION

In the present work, the response of each of the five Cameron Highlands populations at F2 to the discriminating dose for *Btk* and *Bta* suggested the presence of resistant individuals (in relation to the SS), particularly

**TABLE 2**

Relative Residual Toxicity of *Bt* subsp. *kurstaki*, *Bt* subsp. *aizawai* and Abamectin, against an Insecticide-Susceptible Laboratory Strain (ROTH) and Four Malaysian Field Strains of *Plutella xylostella* in Leaf-Dip Bioassays[a]

| *Insecticide* | *Strain*[b] | $LC_{50}$[c] | *95% FL* | *Slope* (±*SE*) | *TR*[d] | *Df*[e] |
|---|---|---|---|---|---|---|
| *Bt* subsp. *kurstaki* | ROTH | 0·047 | 0·017–0·095 | 2·00 (±0·30) | — | 5 |
| | SERD 2 | 0·679 | 0·448–0·983 | 1·97 (±0·33) | 14 | 5 |
| | TRATA | 0·330 | 0·219–0·459 | 3·69 (±0·82) | 7 | 4 |
| | KT | 0·158 | 0·027–0·307 | 1·86 (±0·53) | 3 | 4 |
| | HB | 0·513 | 0·336–0·734 | 2·96 (±0·55) | 11 | 3 |
| *Bt* subsp. *aizawai* | ROTH | 0·092 | 0·063–0·134 | 1·80 (±0·24) | — | 5 |
| | SERD 2 | 0·750 | 0·509–1·052 | 2·50 (±0·50) | 8 | 5 |
| | TRATA | 0·213 | 0·132–0·310 | 2·86 (±0·56) | 2 | 4 |
| | KT | 0·283 | 0·197–0·408 | 3·14 (±0·56) | 3 | 4 |
| | HB | 0·520 | 0·362–0·753 | 2·96 (±0·50) | 6 | 4 |
| Abamectin | ROTH | 0·0004 | 0·0002–0·0007 | 1·33 (±0·22) | — | 6 |
| | SERD 2 | 0·0069 | 0·0053–0·0092 | 2·75 (±0·30) | 17 | 6 |
| | TRATA | 0·0277 | 0·0135–0·0936 | 1·26 (±0·22) | 69 | 4 |
| | KT | 0·0781 | 0·0237–1·2500 | 0·89 (±0·21) | 195 | 6 |
| | HB | 0·0259 | 0·0186–0·0372 | 3·15 (±0·50) | 65 | 6 |

[a] Bioassays with second-instar (abamectin) and third-instar larvae (*Bt* products) at 20 (±2)°C on *B. chinensis* cv. Tip Top; mortality assessed at nine days ($n$ = 120–350). The SERD 2 population was assayed at F10, the other populations at F3.

[b] Strain abbreviations: ROTH = Rothamsted (laboratory: UK); SERD 2 = Serdang 2 (Serdang region, lowland Malaysia); TRATA = Tanah Rata (Central Zone, Cameron Highlands); KT = Kuala Terla (Northern Zone, Cameron Highlands); HB = Habu (Southern Zone, Cameron Highlands).

[c] Units: $\mu$g AI $ml^{-1}$ for abamectin; iu $mg^{-1}$ for *Bt* products.

[d] Toxicity ratio (TR): $LC_{50}$ value of selected strain compared with ROTH (laboratory) strain.

[e] Degrees of freedom.

to *Btk* (Table 1). The subsequent $LC_{50}$ tests at F3 on three of these populations tended to confirm a greater degree of resistance to *Btk* than to *Bta* in the Tanah Rata and Habu populations (compared with ROTH). However, in the Kuala Terla population (which had the greatest number of surviving individuals in the diagnostic test for *Btk* but relatively few survivors with *Bta*) a low resistance ratio of 3 was obtained for both products (Table 2). This apparent difference between the two assays is even greater if $LC_{50}$ resistance ratios are calculated using the data for SS (Table 1, see footnote) rather than ROTH. In this case, none of the values for *Bta* are significantly ($P > 0{\cdot}01$) different (0·4- to 1·5-fold compared with SS).

Discriminating dose assays are often a more effective means for detecting relatively low frequencies of resistance in populations, since all the individuals are tested at a critical dose, whereas in $LC/LD_{50}$ tests some resistant individuals are likely to be tested at lower, less informative doses.[17] The results obtained in the present discriminating dose assays were certainly more in keeping with anecdotal reports (see below) of reduced efficacy for *Btk* products against *P. xylostella* throughout the Cameron Highlands (Verkerk, R. H. J., unpublished results). This was less the case for *Bta* and it is possible that the SS population may not be a particularly suitable standard, since it appears to be relatively more tolerant to *Bta* compared with *Btk* than is the ROTH population. Certainly, a greater number of individuals from field populations might have been expected to survive if the discriminating dose for *Bta* had been based on the response of the ROTH population.

It is possible that some reversal of resistance may have occurred prior to testing at F2 and/or that the relatively small size of the gene pool in the sample population may have excluded some resistance genes. Such changes may also have contributed to the observed discrepancies between the discriminating dose (at F2) and $LC_{50}$ (F3) test results. The previous history for SERD 2 (F0–F9) was not known, but some decline (*c.*3-fold) in resistance to *Btk* and *Bta* was observed in the unselected sub-population of SERD 2 from F10 to F16 (Table 3). A rapid reversal of resistance to *Btk* (over 5–15 generations) has been reported in some highly resistant (up to 2800-fold) sub-populations of *P. xylostella*.[6]

On the above basis, the observed levels of resistance to *Btk* and *Bta* (14- and 8-fold respectively compared with ROTH) at F10 in the lowland, SERD 2 population

**TABLE 3**

Stability of Residual Toxicity of *Bt* subsp. *kurstaki* (*Btk*), *Bt* subsp. *aizawai* (*Bta*) and Abamectin (Abam.) against the SERD 2 Field Strain of *Plutella xylostella* and Studies on the Selection of Resistance and Cross-Resistance to These Compounds

| *Population (generation)* | *Chemical*[a] | $LC_{50}$[b] *(95% FL)* | *Slope (±SE)* | *TR*[c] | *Df* |
|---|---|---|---|---|---|
| ROTH | *Btk* | 0·047 (0·017–0·095) | 2·00 (±0·30) | — | 5 |
| SERD2 | | | | | |
| F10 | *Btk* | 0·68 (0·45–0·98) | 1·97 (±0·33) | 14 | 5 |
| F14 | *Btk* | 0·30 (0·14–0·56) | 1·35 (±0·29) | 6 | 5 |
| F16 | *Btk* | 0·23 (0·13–0·45) | 1·39 (±0·28) | 5 | 5 |
| SERD 2-*Btk*[d] | | | | | |
| F14 | *Btk* | 6·37 (3·76–14·4) | 2·37 (±0·36) | 136 (21) | 5 |
| F16 | *Btk* | 10·0 (6·55–16·5) | 2·09 (±0·34) | 213 (43) | 5 |
| F16 | *Bta* | 0·16 (0·09–0·28) | 1·87 (±0·41) | 2 (0·5) | 6 |
| F16 | Abam. | 0·002 (0·002–0·004) | 2·73 (±0·47) | 5 (1) | 5 |
| ROTH | *Bta* | 0·092 (0·063–0·134) | 1·80 (±0·24) | — | 5 |
| SERD 2 | | | | | |
| F10 | *Bta* | 0·75 (0·51–1·05) | 2·50 (±0·50) | 8 | 5 |
| F14 | *Bta* | 0·48 (0·25–1·03) | 1·28 (±0·28) | 5 | 5 |
| F16 | *Bta* | 0·31 (0·21–0·45) | 2·60 (±0·38) | 3 | 5 |
| SERD 2-*Bta*[d] | | | | | |
| F14 | *Bta* | 1·72 (0·89–5·53) | 1·39 (±0·29) | 19 (4) | 5 |
| F16 | *Bta* | 2·37 (1·36–5·04) | 1·52 (±0·31) | 26 (8) | 6 |
| F16 | *Btk* | 0·83 (0·50–1·36) | 2·02 (±0·42) | 18 (4) | 5 |
| ROTH | Abam. | 0·0004 (0·0002–0·0007) | 1·37 (±0·22) | — | 6 |
| SERD 2 | | | | | |
| F10 | Abam. | 0·007 (0·005–0·009) | 2·75 (±0·30) | 17 | 6 |
| F14 | Abam. | 0·003 (0·002–0·004) | 3·23 (±0·45) | 7 | 5 |
| F16 | Abam. | 0·002 (0·001–0·003) | 2·66 (±0·39) | 5 | 5 |
| SERD 2-Abam.[d] | | | | | |
| F14 | Abam. | 0·013 (0·009–0·022) | 2·43 (±0·35) | 33 (4) | 5 |
| F16 | Abam. | 0·016 (0·011–0·024) | 2·47 (±0·37) | 40 (8) | 5 |
| F19[e] | Abam. | 0·005 (0·004–0·007) | 4·09 (±0·70) | 13 | 5 |
| F16 | *Btk* | 0·19 (0·11–0·30) | 2·24 (+0·45) | 4 (1) | 5 |

[a] Bioassays ($n$ = 180–350) with second-instar (Abam.) or third-instar larvae (*Btk* and *Bta*).

[b] Units: iu $mg^{-1}$ for *Bt* products, $\mu g$ AI $ml^{-1}$ for abamectin.

[c] Toxicity ratio (TR) compared with equivalent $LC_{50}$ value for the ROTH strain (TR compared with unselected SERD 2 at equivalent generation).

[d] Selected (F11–F15)

[e] Without selection pressure from F17 to F18.

of *P. xylostella* (Table 2) were of particular interest, and subsequent selection studies with SERD 2 confirmed the presence of high levels of resistance to *Btk* and moderate levels of resistance to *Bta* (Table 3) with corresponding realized heritability ($h^2$) values of 0·69 and 0·27 respectively (Table 5). These values are greater than any in other published selection studies with *Bt* against *P. xylostella*, and the upper figure is comparable with the highest estimates for other pesticides against this species.[5,13] Although extrapolation of these laboratory observations to field populations is problematic,[18] the potential for *Bt* resistance in Malaysia is clearly of considerable cause for concern.

Studies on resistance to *Bt* in *P. xylostella* populations have generally shown little or no cross-resistance between products or specific toxins.[5] For example, a population from Hawaii with >1000-fold resistance to *Btk* as well as appreciable resistance to four of its constituent crystal toxins was not resistant to CryIC, although extreme resistance to *Btk* was reported to give 3-fold cross-resistance to *Bta*.[18] Other field populations of *P. xylostella* from Malaysia with >100-fold resistance to *Btk* were reported to have <10-fold resistance to *Bta*.[8] Changes in midgut membrane, *Bt*-binding receptors have so far been identified as the primary mechanisms of resistance to *Bt* toxins in *P. xylostella*,[5] and these changes tend to be specific to particular crystal proteins.[19] Thus, when reduced binding is the major mechanism of resistance, cross-resistance would be expected only between strains or subsp. of *Bt* that share toxins or where toxins have common binding sites.[5]

**TABLE 4**
Stability of Residual Toxicity of Teflubenzuron (Tefb.) against Second-Instar Larvae of the SERD 2 Field Strain of *Plutella xylostella* and Selection of Resistance

| *Strain (Generation)* | $LC_{50}$(*μg AI ml*$^{-1}$) (*95% F.L.*)[a] | *Slope* (±*SE*) | *TR*[b] | *Df* |
|---|---|---|---|---|
| ROTH (Fn + 1) | 0·08 (0·04–0·13) | 1·90 (±0·35) | — | 6 |
| SERD 2 | | | | |
| F10 | 1·46 (1·06–2·06) | 2·21 (±0·28) | 18 | 5 |
| F14 | 1·18 (0·84–1·71) | 2·81 (±0·39) | 15 | 5 |
| F16 | 0·88 (0·68–1·22) | 3·99 (±0·54) | 11 | 5 |
| SERD 2-Tefb[c] | | | | |
| F14 | 12·0 (6·84–44·5) | 2·24 (±0·58) | 150 (10) | 5 |
| F16 | 42·0 (23·6–82·5) | 1·44 (±0·29) | 525 (48) | 5 |

[a] Mortality assessed at day 13; $n$ = 180–350.
[b] Toxicity ratio compared with $LC_{50}$ for the ROTH strain (TR compared with SERD 2 at equivalent generation).
[c] Selected F11–F15).

The apparent low level of cross-resistance to *Btk* in the *Bta*-selected sub-population of SERD 2 (Table 3) may thus involve reduced binding of CryIA proteins which are common to both these subsp. of *Bt*. The apparent absence of cross-resistance to *Bta* in the *Btk*-selected sub-population is less easy to explain. This may reflect a failure to detect very low levels of cross-resistance (selection of resistance to *Bta* being less than to *Btk* within the SERD 2 population). However, the sensitivity to *Bta* actually appeared to increase slightly, although not significantly ($P > 0{\cdot}01$), in the *Btk*-selected sub-population (Table 3), which suggests that there may be an alternative explanation. For example, reduced binding of CryIA proteins in a *Btk*-selected (CryIA-resistant) population of the Indian meal moth, *Plodia interpunctella* Hübner, was found to be accompanied by an apparent increase in binding of CryIC and a corresponding increased sensitivity (*c.*4-fold) to the latter protein.[20] Further studies are clearly required to determine the contribution of different Cry proteins, including their synergistic and antagonistic interactions, to the overall toxicity of *Bt* Isolates in susceptible and resistant populations of insects.

Recent studies on a field population of *P. xylostella* have also suggested that, apart from reduced binding, other biochemical mechanisms are involved in resistance to *Bt*.[21] Such mechanisms, involving pre- or post-binding events,[5] may not be so specific and could lead to more broad-spectrum resistance to *Bt* products, as implied by studies on laboratory-selected populations of *Heliothis virescens* F.[22,23]

The lack of cross-resistance between *Btk* and abamectin found in the present study (Table 3) is in keeping with a general absence of cross-resistance between conventional pesticides and *Bt*,[5] a reflection of the very different resistance mechanisms they are known to generate.

All four field populations tested appeared to show resistance to abamectin (TR = 17 to 195-fold compared with ROTH), most notably in the Kuala Terla population (Table 2). This was in contrast to previous reports, where resistance to abamectin was not found in any of the Malaysian lowland or highland populations of *P. xylostella* examined.[8,24] The present study may be the first reported case of marked resistance to abamectin in a field population of *P. xylostella*, although some

**TABLE 5**
Estimation of Realised Heritability ($h^2$)[18] of Resistance from Laboratory Selection Experiments (over Five Generations) with SERD 2 Strain of *Plutella xylostella*

| | *Estimate of mean response per generation*[a] | | | *Estimate of mean selection differential per generation*[b] | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *Selected strain* | *Initial* $LC_{50}$ (*log*) | *Final* $LC_{50}$ (*log*) | R | P | i | *Initial slope* | *Final slope* | $\sigma_p$ | S | $h^2$ |
| *Btk* | −0·167 | 1 | 0·233 | 57 | 0·689 | 1·97 | 2·09 | 0·493 | 0·340 | 0·69 |
| *Bta* | −0·125 | 0·375 | 0·100 | 54 | 0·735 | 2·50 | 1·52 | 0·498 | 0·366 | 0·27 |
| Abam. | −2·155 | −1·796 | 0·072 | 58 | 0·674 | 2·75 | 2·47 | 0·383 | 0·258 | 0·28 |
| Tefb. | 0·164 | 1·623 | 0·292 | 56 | 0·704 | 2·21 | 1·44 | 0·548 | 0·386 | 0·76 |

[a] R = response to selection.[18]
[b] P = Percentage of population surviving selection; i = intensity of selection; $\sigma_p$ = phenotypic standard deviation; s = selection differential.[18]

evidence of cross-resistance to abamectin was reported in a multi-insecticide-resistant population collected in Thailand in 1982 prior to the introduction of this product.[9] The apparent decline (*c.*3-fold) in resistance to abamectin in the unselected sub-population of SERD 2 from F10 to F16 and in the selected sub-population from F16 to F19 (Table 3), suggests the level of resistance in a freshly collected field population of SERD 2 would have been more comparable with that of the Cameron Highlands populations tested. The estimate for $h^2$ for selection of resistance by abamectin in SERD 2 (Table 5) was almost identical to the value obtained for *Bta* and implies a relatively low rate of selection compared with *Btk*.

The high levels of resistance to teflubenzuron (Table 4) which could be rapidly selected in the SERD 2 population ($h^2 = 0.76$; Table 5) are in agreement with studies on a population of *P. xylostella* collected in the same area in 1992.[25] This suggests that resistance would increase rapidly if this compound were re-introduced on a large scale in the near future.

The present study has shown that resistance to *Btk* and abamectin, and to a lesser extent *Bta*, appears to be widespread in the Cameron Highlands vegetable-growing area and is also present in at least one lowland area of Malaysia. Without an immediate and effective insecticide resistance management (IRM) strategy,[5,26] at least two of the three products which have so far proved highly compatible with an IPM approach involving biological control of *P. xylostella* may be lost. The apparent, relatively high frequency of resistance to *Btk* and *Bta* in field populations of *P. xylostella* in Malaysia suggests that mixtures of these toxins (see Introduction) are unlikely to remain effective[26] and immediate measures aimed at reducing the usage of *Btk* and *Bta* without the re-introduction of broad-spectrum insecticides are necessary. The lack of cross-resistance between *Bt* and abamectin offers only a partial solution.

Historically, new compounds have been introduced sequentially for control of *P. xylostella* against a background of existing resistance problems and each in turn has succumbed to resistance and eventually become ineffective. The fate of the relatively selective acylurea insect growth regulator, teflubenzuron, is a recent example.[25] Until new selective compounds are introduced into a managed system, probably involving the alternation of a number of dissimilar products,[26] severe resistance problems will continue and IPM systems for *P. xylostella* will remain at best only partially successful.

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